EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 14105

March 2023

ICS 67.200.10

Will supersede EN 14105:2020

English Version

Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of free and total glycerol and mono-, di-, triglyceride contents

Produits dérivés des corps gras - Esters méthyliques d'acides gras (EMAG) - Détermination de la teneur en glycérols libre et total et en mono-, di- et triglycérides

Erzeugnisse aus pflanzlichen und tierischen Fetten und Ölen - Fettsäure-Methylester (FAME) - Bestimmung des Gehaltes an freiem und Gesamtglycerin und Mono-, Di- und Triglyceriden

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 307.

If this draft becomes a European Standard, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

This draft European Standard was established by CEN in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and United Kingdom.

Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

Warning : This document is not a European Standard. It is distributed for review and comments. It is subject to change without notice and shall not be referred to as a European Standard.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Ref. No. prEN 14105:2023 E

Contents

Page

European foreword

This document (prEN 14105:2023) has been prepared by Technical Committee CEN/TC 307 “Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis”, the secretariat of which is held by AFNOR.

This document is currently submitted to the CEN Enquiry.

This document will supersede EN 14105:2020.

In comparison with the previous edition, the following technical modifications have been made:

— the weighing accuracy is specified.

1 Scope

This document specifies a method to determine the free glycerol and residual mono-, di- and triglyceride contents in fatty acid methyl esters (FAME). The total glycerol content is then calculated from the obtained results.

Under the conditions described, the quantification limits are 0,001 % (*m*/*m*) for free glycerol, 0,10 % (*m*/*m*) for all glycerides (mono-, di- and tri-). This method is suitable for FAME prepared from rapeseed, sunflower, soybean, palm, animal oils and fats and mixture of them. It is not suitable for FAME produced from or containing coconut and palm kernel oils derivatives because of overlapping of different glyceride peaks.

NOTE 1 For the purposes of this document, the term "% (*m*/*m*)" is used to represent the mass fraction.

NOTE 2 Under the common EN 14105 GC conditions, squalene can coelute with alpha glycerol monostearate. If the presence of squalene is suspected, EN 17057 can be used to discriminate between squalene and glycerol monostearate.

WARNING — The use of this document can involve hazardous materials, operations and equipment. This document does not purport to address all of the safety problems associated with its use. It is the responsibility of users of this document to take appropriate measures to ensure the safety and health of personnel prior to application of the standard, and fulfil statutory and regulatory requirements for this purpose.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 5555, *Animal and vegetable fats and oils - Sampling (ISO 5555)*

EN ISO 3170, *Petroleum liquids - Manual sampling (ISO 3170)*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principle

The glycerol and the mono- and diglycerides are transformed into more volatile and stable silyl derivatives in presence of pyridine and of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA).

The sample after silylation is analysed by gas chromatography on a short capillary column with thin film thickness, with an on-column injector or equivalent device, and flame ionization detection.

After a calibration procedure, the quantification of glycerol is carried out in presence of the internal standard 1,2,4-butanetriol.

Mono-, di- and triglycerides are directly evaluated in presence of an internal standard for each glyceride category:

— glyceryl monononadecanoate (Mono C19) for monoglycerides;

— glyceryl dinonadecanoate (Di C38) for diglycerides;

— glyceryl trinonadecanoate (Tri C57) for triglycerides.

5 Chemicals

Use only chemicals of recognized analytical grade, unless otherwise specified.

5.1 N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA).

5.2 Pyridine, max. 0,1 % water, stored on molecular sieve.

Pyridine silyl grade (5.10) can also be used.

5.3 Tetrahydrofurane (THF).

5.4 n-Heptane.

5.5 Glycerol.

5.6 1,2,4-Butanetriol.

5.7 1-Glyceryl monononadecanoate (Mono C19).

5.8 1-3 Glyceryl dinonadecanoate (Di C38).

5.9 Glyceryl trinonadecanoate (Tri C57).

5.10 Pyridine, silyl grade.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Gas chromatograph, equipped with an on-column injector or equivalent device, a temperature-programmable oven and a flame ionization detector.

6.2 Capillary column, capable of being programmed up to 400 °C ("high temperature" type) for which the following characteristics are advised:

— 100 % dimethylpolysiloxane or 95 % dimethyl-5 % diphenylpolysiloxane stationary phase;

— length 15 m;

— internal diameter 0,32 mm;

— film thickness 0,1 µm.

6.3 Volumetric flask, 50 ml capacity, Grade A.

6.4 Volumetric flasks, 20 ml capacity, Grade A.

6.5 Volumetric flasks, 10 ml capacity, Grade A.

6.6 Screw-cap vials with PTFE-faced septa, 10 ml capacity.

6.7 Precision pipette, 1 ml capacity.

6.8 Precision pipette or syringe, 100 µl capacity.

6.9 Precision pipette or syringe, 500 µl capacity.

6.10 Graduated cylinder, 10 ml capacity.

6.11 Analytical balance, with a weighing accuracy of ± 1 mg or better and a readability of ± 0,1 mg or better.

6.12 Carrier gas, hydrogen or helium.

6.13 Auxiliary gases, such as air, hydrogen and nitrogen.

7 Preparation of solutions

7.1 1,2,4-Butanetriol stock solution, 1 mg/ml

Weigh, to the nearest 0,1 mg, 40 mg to 60 mg of 1,2,4-butanetriol (5.6) in a 50 ml volumetric flask (6.3) and fill up to the mark with pyridine (5.2 or 5.10).

7.2 Glycerol stock solution, 0,5 mg/ml

Weigh, to the nearest 0,1 mg, 40 mg to 60 mg of glycerol (5.5) in a 10 ml volumetric flask (6.5) and fill up to the mark with pyridine (5.2 or 5.10). Transfer 1 ml of this solution into a 10 ml volumetric flask (6.5) using a pipette (6.7) and fill up to the mark with pyridine (5.2 or 5.10).

7.3 Standard glycerides stock solution, 2,5 mg/ml

For each reference glyceride, monononadecanoate (5.7), dinonadecanoate (5.8) and trinonadecanoate (5.9), weigh, to the nearest 0,1 mg, 40 mg to 60 mg in a unique 20 ml volumetric flask (6.4) and fill up to the mark with tetrahydrofurane (5.3).

The solution shall be perfectly transparent at ambient temperature. After storage in a refrigerator at 4 °C the solution can show a precipitate that shall re-dissolve spontaneously when restored at ambient temperature, without any external heating.

NOTE If stored at 4 °C the solution is stable for almost 3 months.

7.4 Commercial mixture of monoglycerides

Made up of mono-palmitoylglycerol (monopalmitin), mono-stearoylglycerol (monostearin) and of mono oleoylglycerol (monoolein), present in quantities having an identical mass.

Prepare a stock solution of this mixture by weighing approximately 100 mg in a 10 ml volumetric flask (6.5) and fill up to the mark with pyridine (5.2 or 5.10).

This solution may be used to locate the relevant peaks in GC paths.